particles[17]. It has been proposed that during assembly of a retrovirus particle, a localized high concentration of gag–pol precursor leads to a conformational change of the proteinase (possibly dimer formation), autocatalytic cleavage of the gag–pol precursor, release of active proteinase into the interior of the virion, and further proteinase-mediated cleavage of the precursors[10,13]. This mechanism affords a potentially critical advantage to virus assembly: by being anchored to the inside of the membrane, proteinase is prevented from diffusing away from the site of assembly until virion formation is well under way and the enzyme is entrapped in the virion.

Designing an inhibitor that is incorporated into the HIV virion at levels sufficient to inhibit the free form of proteinase (p12) may be more difficult than delivering a drug to the cytoplasm. Alternatively, it may be necessary to design and synthesize agents that bind the gag–pol precursor and prevent proper assembly and/or the initial catalytic event. The research group at Merck has recently analysed purified HIV proteinase on urea gradient gels and demonstrated that the dimer is the only stable, folded form of the enzyme at pH 4.0 (C. DiInni and P. Darke, pers. commun.), supporting the hypothesis that inhibition of dimer formation is a potential target for antiviral action.

□ □ □

All promising approaches must be investigated in the search for improved therapies to treat HIV-infected individuals. The proteinase is one of several potential therapeutic targets. Although this article has referred to research on the HIV-1 proteinase, the HIV-2 proteinase presents a similarly appealing target. Several groups within both the public and private sectors and with a wide range of expertise are currently focusing their research efforts in this direction. The recent elucidation of the crystallographic structure of the HIV-1 proteinase represents an important step, but challenging questions remain to be answered before effective and safe drugs against HIV proteinase (or other viral and cellular proteinases) can be developed.

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References

1 Krausslich, H. G., Oroszlan, S. and Wimmer, E. (1989) *Current Communications in Molecular Biology: Viral Proteinases as Targets for Chemotherapy*, Cold Spring Harbor Laboratory Press
2 Krausslich, H. G. and Wimmer, E. (1988) *Annu. Rev. Biochem.* 57, 701–754
3 Roberts, M. M. and Oroszlan, S. (1989) *Biochem. Biophys. Res. Commun.* 160, 486–494
4 Kohl, N. E. *et al.* (1988) *Proc. Natl Acad. Sci. USA* 85, 4686–4690
5 Le Grice, S. F. J., Mills, J. and Mous, J. (1988) *EMBO J.* 7, 2547–2553
6 Seelmeier, S., Schmidt, S. H., Turk, V. and Von der Helm, K. (1988) *Proc. Natl Acad. Sci. USA* 85, 6612–6616
7 Sham, H. L. *et al.* (1988) *J. Med. Chem.* 31, 284–295
8 Billich, S. *et al.* (1988) *J. Biol. Chem.* 263, 17905–17908
9 Kotler, M., Katz, R. A., Dahno, W., Leis, J. and Skalka, A. M. (1988) *Proc. Natl Acad. Sci. USA* 85, 4185–4189
10 Krausslich, H. G. *et al.* (1989) *Proc. Natl Acad. Sci. USA* 86, 807–811
11 Loeb, D. D., Hutchinson, C. A., III, Edgell, M. H., Farmerie, W. G. and Swanstrom, R. (1989) *J. Virol.* 63, 111–121
12 Debouck, C. *et al.* (1987) *Proc. Natl Acad. Sci. USA* 84, 8903–8906
13 Farmerie, W. G. *et al.* (1987) *Science* 236, 305–308
14 Navia, M. A. *et al.* (1989) *Nature* 337, 615–620
15 Pearl, L. H. and Taylor, W. R. (1987) *Nature* 329, 351–354
16 DesJarlais, R. L. *et al.* (1988) *J. Med. Chem.* 31, 722–729
17 Witte, O. and Baltimore, D. (1978) *J. Virol.* 26, 750–761
18 Leis, J. *et al.* (1988) *J. Virol.* 62, 1808–1809

5-HT_{1C} receptor activation: a key step in the initiation of migraine?

'When all has been said that can be, mystery still envelops the mechanism of migraine.'
W. Gowers 1893

In June 1988 Brewerton and his coworkers[1] published the remarkable observation that *m*-chlorophenylpiperazine (*m*-CPP), a major metabolite of the antidepressant trazodone and a selective 5-HT_1 receptor agonist, can induce migraine-like headaches in humans. Thirty-seven female patients with eating disorders and 15 age-matched female control subjects were studied. Of the patients, 26 suffered from bulimia and 11 suffered from anorexia nervosa. The subjects received on three separate occasions, under randomized, double-blind conditions, either *m*-CPP (0.5 mg kg^{-1} orally, a commonly used dose in both clinical and preclinical investigations), placebo, or L-tryptophan (100 mg kg^{-1} i.v.).

Severe headaches occurred in 28 out of the 52 subjects (54%) after *m*-CPP, but were not reported by any of the subjects after placebo or L-tryptophan. The headaches tended to be delayed in onset, occurring 4–12 h after intake of *m*-CPP. Headaches were reported with similar frequency by the patients with eating disorders and the control subjects.

The severe headaches had many features typical of migraine without aura including nausea (86%), photophobia (86%), throbbing (75%), unilaterality (32%) and involuntary vomiting (7%). Interestingly 20 of the 52 subjects had a personal or family history of migraine, and of these 18 (90%) developed severe headache. In seven out of the eight subjects with a personal history of migraine, the *m*-CPP-induced headaches were described as essentially indistinguishable from their naturally occurring migraine headaches, except possibly for the absence of a prodrome. Peak plasma concentrations of *m*-CPP occurred 2–3 h after administration, unlike the time of peak occurrence of headache, which was 8–12 h after drug intake. Despite this lack of temporal correlation, headache ratings were significantly correlated with the peak plasma concentrations of *m*-CPP.

In summary, the findings of Brewerton *et al.* demonstrate unequivocally that typical migraine headaches may be induced by *m*-CPP, a 5-HT_1 receptor agonist.

TABLE I. Effects of *m*-chlorophenylpiperazine at 5-HT$_1$ binding sites and in functional tests

Receptor subtype	Binding (pK_d)	Test	Function (pEC_{50})	Efficacy (% 5-HT)
5-HT$_{1A}$	6.6	↓ adenylate cyclase	5.9	40
5-HT$_{1B}$	6.6	↓ adenylate cyclase	6.5	60
5-HT$_{1C}$	7.8	↑ PI	6.9	65
5-HT$_{1D}$	6.8	↓ adenylate cyclase	[5.1]	~30

[ ] indicates pK_b. Data from Refs 9 and 10.

This important finding should be seen both in a historical perspective and in the context of the recent advances in our understanding of 5-HT$_1$ receptor subtypes and their function.

A role for 5-HT in the pathogenesis of migraine has long been suspected (reviewed in Refs 2 and 3). The evidence includes the fact that attacks, indistinguishable from spontaneous migraine, can be triggered in migraineurs by drugs whose primary action involves the mobilization of endogeneous 5-HT from both neuronal and non-neuronal sites. Thus, low doses (0.5–2.5 mg) of reserpine are consistently active in this respect[4–6]. Moreover, that a release of 5-HT, rather than its depletion, is the important mechanism is strongly suggested by the fact that such reserpine-induced headaches can be prevented by prior treatment with methysergide[7]. Furthermore, the highly selective 5-HT releasing agent, fenfluramine, has been shown in a well-controlled double-blind trial to trigger migraine headaches in a majority of migraineurs; the same dose was ineffective in non-migraineurs[8].

The Brewerton *et al.* observation becomes still more interesting when one considers the recent evidence that *m*-CPP is a rather selective 5-HT$_{1C}$ receptor agonist: in *in-vitro* studies using radioligand binding and second messenger responses, Hoyer and his colleagues[9,10] have demonstrated that *m*-CPP has reasonably potent agonist properties at 5-HT$_{1B}$ and 5-HT$_{1C}$ receptors (Table I). Since 5-HT$_{1B}$ sites appear to be rodent specific[11] it is likely that *m*-CPP acts rather selectively at 5-HT$_{1C}$ receptors in humans, especially at the low concentrations such as those reported in the plasma by Brewerton *et al.* (peak concentration 1.8×10^{-7} M).

Accumulating *in-vivo* evidence from behavioural studies also supports an important rol f r 5-HT$_{1C}$ receptors in the mod of action of *m*-CPP. Kenn tt and Curzon[12,13] have provided evidence that hypophagia in food-deprived rats, and hypoactivity in rats in response to *m*-CPP may be mediated via stimulation of 5-HT$_{1C}$ receptors. Similarly, anxiogenic-like effects of *m*-CPP in animal models can be reversed by 5-HT$_{1C}$ receptor antagonists[14]. Finally, Neill and Cooper have shown that *m*-CPP may act through 5-HT$_{1C}$ receptors to decrease consumption of both hypertonic saline in thirsty rats[15] and palatable food in non-deprived rats[16].

The plot thickens further when it becomes evident that fenfluramine may also exert behavioural effects through activation of 5-HT$_{1C}$ receptors. Cunningham *et al.*[17] have shown that the ability of rats to discriminate the 5-HT receptor agonist MK-212 from saline is almost certainly due to 5-HT$_{1C}$ receptor activation. Significantly both *m*-CPP and fenfluramine mimicked MK-212 in this test, whereas many other, less selective, 5-HT receptor agonists did not. Neill and Cooper have also recently provided evidence for a role for 5-HT$_{1C}$ receptors in mediating the anorectic effect of fenfluramine[18].

Putting all the above evidence together, the conclusion emerges that increased activation of 5-HT$_{1C}$ receptors may be a means to trigger migraine. If this is correct, 5-HT$_{1C}$ receptor antagonists should be effective as prophylactic agents in migraine.

They are indeed. Methysergide and pizotifen, both potent 5-HT$_{1C}$ receptor antagonists (albeit nonselective; see Table II) are well established as effective migraine prophylactic agents[19]. Cyproheptadin , another 5-HT$_{1C}$ receptor antagonist is also claimed to be effectiv in this respect[20], as is mianserin[21]. Although all these agents are also potent antagonists at 5-HT$_2$ receptors[22], two pieces of evidence favour the importance of the 5-HT$_{1C}$ receptor rather than the 5-HT$_2$ receptor in the triggering of migraine attacks. Firstly, *m*-CPP has only weak affinity for 5-HT$_2$ receptors[10] and is an antagonist at these sites[23–25]. Secondly, ketanserin, which has good selectivity for 5-HT$_2$ sites over 5-HT$_{1C}$ sites (see Table II) appears, at least in a preliminary study[26] to be only weakly active as a migraine prophylactic agent. The single relevant common denominator of the main migraine prophylactic drugs may therefore be 5-HT$_{1C}$ receptor antagonism (Table II).

As to where and how stimulation of 5-HT$_{1C}$ receptors may lead to migraine attacks, one must return to current '5-HT-based' theories of migraine (summarized recently in Ref. 27). The most plausible current scenario is that an inappropriate increase or fluctuation in the activation of the 5-HT neurones of the brain stem, by activation of 5-HT$_{1C}$ receptors, would trigger the 'sterile inflammatory response' around blood vessels, which has been claimed to play a role in the pathophysiology of acute migraine[2,3,28]. This suggestion is supported by the fact that 5-HT can generate pro-inflammatory mediators from vascular smooth muscle[29] and that the response can be blocked by 5-HT$_{1C}$/5-HT$_2$ receptor antagonists[29,30].

□ □ □

Through whatever mechanism, we believe 5-HT$_{1C}$ receptor stimulation to be an important step in the pathogenesis of migraine attacks, and that just a little less

TABLE II. Affinities (pK_d) of drugs for 5-HT receptor subtypes

	5-HT$_{1A}$	5-HT$_{1B}$	5-HT$_{1C}$	5-HT$_{1D}$	5-HT$_2$
Methysergide	7.6	6.8	8.6	8.4	8.6
Pizotifen	6.2	5.5	8.1	5.6	7.8
Cyproheptadine	6.5	5.3	7.9	n.d.	8.5
Mianserin	6.0	5.2	8.0	6.4	8.1
Ketanserin	5.9	5.7	7.0	6.0	8.9

n.d.–no data. Data from Ref. 10 (radioligand binding assays).

mystery *'envelops the mechanism of migraine'*.

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References

1 Brewerton, T. D., Murphy, D. L., Mueller, E. A. and Jimerson, D. C. (1988) *Clin. Pharmacol. Ther.* 43, 605–609
2 Fozard, J. R. (1982) in *Drugs and Platelets* (Van Zwieten, P. A. and Schönbaum, E., eds) (*Progress in Pharmacology* Vol. 4), pp. 135–146, Fischer
3 Fozard, J. R. (1985) in *Vascular Neuroeffector Mechanisms* (Bevan, J. A. *et al.*, eds), pp. 321–328, Elsevier
4 Kimball, R. W., Friedman, A. P. and Vallejo, E. (1960) *Neurology* 10, 107–111
5 Curzon, G., Barrie, M. and Wilkinson, M. I. P. (1969) *J. Neurol. Neurosurg. Psychiatry* 32, 555–561
6 Nappi, G., Savoldi, F., Bono, G. and Martignoni, E. (1979) *Headache* 19, 273–277
7 Carroll, J. D. and Hilton, B. P. (1974) *Headache* 14, 149–156
8 Del Bene, E. *et al.* (1977) in *Headache: New Vistas* (Sicuteri, F., ed.), pp. 101–109, Biomedical Press
9 Hoyer, D. and Schoeffter, P. (1989) *Br. J. Pharmacol.* 96, 9P
10 Hoyer, D. (1989) in *The Peripheral Actions of 5-Hydroxytryptamine* (Fozard, J. R., ed.), pp. 72–99, Oxford University Press
11 Palacios, J. M. and Dietl, M. M. (1988) in *The Serotonin Receptors* (Sanders-Bush, E., ed.), pp. 89–138, Humana
12 Kennett, G. A. and Curzon, G. (1988) *Br. J. Pharmacol.* 94, 137–147
13 Kennett, G. A. and Curzon, G. (1988) *Psychopharmacology* 96, 93–100
14 Kennett, G. A., Whitton, P., Shah, K. and Curzon, G. (1989) *Eur. J. Pharmacol.* 164, 445–454
15 Neill, J. C. and Cooper, S. J. *Psychopharmacology* (in press)
16 Neill, J. C. and Cooper, S. J. *Br. J. Pharmacol.* (Abstr.) (in press)
17 Cunningham, K. A., Callahan, P. M. and Appel, J. B. (1986) *Psychopharmacology* 90, 193–197
18 Neill, J. C. and Cooper, S. J. (1989) *Psychopharmacology* 97, 213–218
19 Fozard, J. R. (1988) in *The Management of Headache* (Clifford Rose, F., ed.), pp. 97–114
20 Peatfield, R. (1986) *Headache*, Springer
21 Monro, P., Swade, C. and Coppen, A. (1985) *Acta Psychiatr. Scand.* 72 (Suppl. 320), 98–103
22 Van Nueten, J. M. (1984) in *The Pharmacological Basis of Migraine Therapy* (Amery, W. K., Van Nueten, J. M. and Wauquier, A., eds), pp. 19–35, Pitman
23 Cohen, M. L. and Fuller, R. W. (1983) *Life Sci.* 32, 711–718
24 Cohen, M. L., Kurz, K. D. and Fuller, R. W. (1987) *Clin. Exp. Theory Pract.* A9, 1549–1565
25 Conn, P. J. and Sanders-Bush, E. (1987) *J. Pharmacol. Exp. Ther.* 242, 552–557
26 Winther, K. (1985) *Cephalagia* 5 (Suppl. 3), 402–403
27 Glover, V. and Sandler, M. (1989) *Trends Pharmacol. Sci.* 10, 1–3
28 Moskowitz, M. A. (1984) *Ann. Neurol.* 6, 157–168
29 Coughlin, S. R., Moskowitz, M. A., Antoniades, H. N. and Levine, L. (1981) *Proc. Natl Acad. Sci. USA* 78, 7134–7138
30 Coughlin, S. R., Moskowitz, M. A. and Levine, L. (1984) *Biochem. Pharmacol.* 33, 692–695

MK-212: 1-(6-chloro-2-pyrazino)piperazine

CURRENT TECHNOLOGY

Identification of human serum cholinesterase variants using the polymerase chain reaction amplification technique

The polymerase chain reaction (PCR) is a newly developed techniqu for selectively amplifying RNA r DNA sequences[1] (see Box). Variations of the technique are proving to be powerful tools in fields as diverse as cancer and population biology. In pharmacology, PCR will allow rapid identification of point mutations or deletions of DNA responsible for variant forms or structural deficiencies in receptors and other proteins of pharmacological interest which are present in low abundance. It has already been useful in the analysis of mutations responsible for variants of human serum cholinesterase.

Applications of PCR

PCR amplification[2,3] is a remarkably powerful technique. If the human genome were 1000 km long, and contained about 100 000 genes, the average gene of 50 kbp would occupy about 10 m. A 300 bp segment amplified by PCR would represent less than 10 cm. The repeated cycles of annealing, primer extension and heat denaturation (see Box) are an extremely efficient process. Millions of copies of the selected segment are generated, and the amplified DNA can easily be seen in agarose gels stained with ethidium bromide. Regions of interest can be sequenced directly using specific sequencing primers. Alternatively, amplification primers can be constructed with synthetic restriction sites at their 5′ ends to allow the amplified s gments to be inserted into suitable vectors, such as M13 phage, for single-strand sequencing[4]. This is particularly useful for long amplified segments and may also be advantageous in searching for n w point mutations within heterozygous samples, where very clean DNA is needed to reduce confusion with shadow bands.

The major limitation of the PCR technique is a direct consequence of its remarkable sensitivity: contamination of the sample by even minute amounts of any DNA recognized by the amplification primers can result in very efficient amplification of the unwanted material. Precautions must be taken to reduce the possibilities for contamination as much as possible, and controls (without template DNA) must be included routinely.

PCR can be used for the analysis of DNA mutations that cannot readily be detected in any other way (for example, mutations producing an alternative codon for the same amino acid, so that the enzyme is unchanged). Quantitative structural variants may result from mutations affecting the half-life of the enzyme, without producing any changes in the quality of its catalytic activity. In fact, it may be impossible to identify these variants by any test based upon kinetic properties, such as a response to competitiv inhibitors. PCR also provides a simple means of diagnosing such alleles in heterozygous individuals. Presumably, a structural change confers greater instability,